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Chapter 1 BASIC CONCEPTS IN PARTICLE CHARACTERIZATION

1. Particles

What is a particle? According to Webster's Dictionary, a particle is "a minute quantity or fragment" or "a relatively small or the smallest discrete portion or amount of something." Because the word "small" is relative to "something," a particle can be as small as a quark or as large as the sun (Fig. 1.1). In the vast universe, the sun is just a small particle! Thus, the range of sciences and technologies for studying particles can be as broad as we can imagine, from astrophysics to nuclear physics. Someone who knows nothing of particle characterization may think that it is a part of particle physics and that all particle physicists actually are studying about micron sized particles. Therefore, we have to define the type of particles in which we are interested.

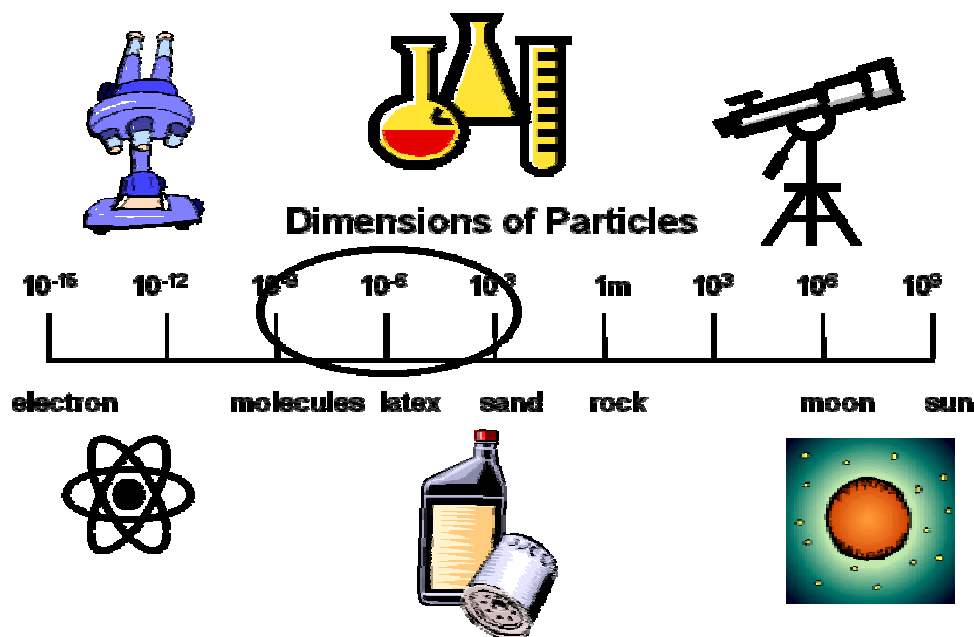


Figure 1.1 Dimension of Particles

"Fine Particles" is a term normally used for particles ranging from a few nanometers to a few millimeters. Particles may exist in some very different forms. They can be synthetic or natural macromolecules in linear forms or in networks, such as proteins, gels, DNAs, latexes, etc.; they can also be ensembles of small inorganic or organic molecules, or even pieces of "space", such as coated bubbles. More typically, they may be just miniscule pieces of bulk materials, such as metal oxides, sugar, pharmaceutical powders, paint, or even the non-dairy creamer we put in coffee.

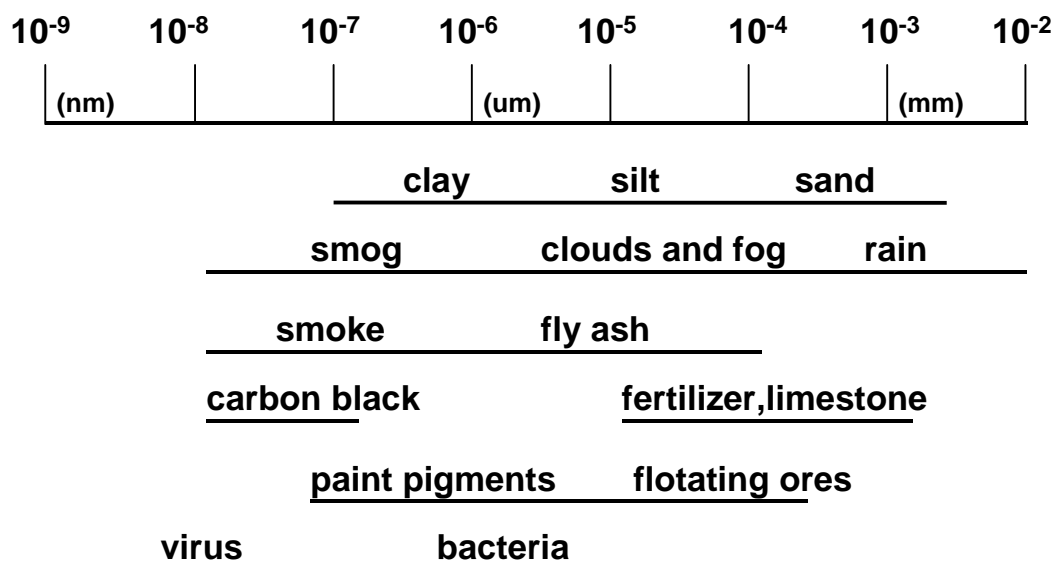


Figure 1.2. Dimension of Industrial Particles

The study of particles in the size range shown in Fig 1.2 is the main subject of Particle Characterization. For particles in this size range, there are two properties, which distinguish them from bulk materials:

1. In a system there is a large number of particles. Each individual particle may have different physical or chemical properties if the material is not homogeneous. The ensemble behavior is usually what is macroscopically observable. The macroscopic properties are derived from contributions of individual particles. If the property we are concerned with is the same for all particles in the system, the system is called "monodisperse." If all or some of the particles in the system have different values for the property of interest, the system is called "polydisperse." Another term, "pausidisperse" is sometimes used to describe situations where there are a few distinct groups within a system. All particles have the same value for the property concerned within each group but different values between the groups.

2. The specific surface area (surface area per unit mass) of such particles is so large that it leads to many significant interfacial phenomena, such as surface interaction with the surrounding medium and neighboring particles. For example, a spherical particle of density 2 g/cm^3 will have a specific surface area of $3 \text{ cm}^2/\text{g}$ when its diameter is 1 cm . The specific surface area will increase to $300 \text{ m}^2/\text{g}$ if the diameter is reduced to 10 nm . This example illustrates how a particle's dimension determines the surface area, which consequently determines the thermodynamics and kinetic stability of a given particulate system.

The above mentioned surface property is especially unique for colloidal particles. The word *colloid* was coined by Thomas Graham in 1861 from Greek roots meaning 'glue-like' and was based on his observation that glue molecules do not pass through a parchment membrane. Thus, *colloid science* is based on the size of the colloidal

unit. Many physical properties besides the one observed by Graham are shown by colloidal systems, that is, systems with at least one dimension less than about a micron. Generally speaking, colloidal particles have dimensions from 10^{-9} m to 10^{-6} m.

2. Particles and Our Society

In the above matrix, colloidal suspensions, aerosols and emulsions are prevalent in many fields and have the largest application in industry or academia. The following is just a short list of fields involved with particulate systems:

- | | |
|------------------------------------|--------------------------|
| ◆ Abrasives | ◆ Fish farming |
| ◆ Biomedical | ◆ Fuel |
| ◆ Biotechnology | ◆ Hydraulic fluids |
| ◆ Beer Industry | ◆ Lubricants |
| ◆ Cell biology | ◆ Metals |
| ◆ Cryobiology | ◆ Marine Biology |
| ◆ Chromatographic material | ◆ Microspheres |
| ◆ Clays | ◆ Paints and pigments |
| ◆ Cosmetics | ◆ Paper industry |
| ◆ Crystals | ◆ Pharmaceuticals |
| ◆ CMP | ◆ Petrochemical Industry |
| ◆ Environmental | ◆ Pesticides |
| ◆ Emulsion | ◆ Photo industry |
| ◆ Electronic Industry | ◆ Oils |
| ◆ Filtration and filter efficiency | ◆ Toners |
| ◆ Food Industry | ◆ Water contamination |

Particle technologies are deeply embedded in our society. Not only are they used as analytical tools in many industries for quality and process control, but also extremely useful in some not so obvious areas like the environmental industry for waste disposal, pollution prevention and emission monitoring. New industries, like biotechnology, are increasingly using particle characterization analyses in both research and production process.

3. Properties of Particle Systems

Here we will discuss mostly the physical or physical chemistry properties of particle systems and pay less attention to their chemical composition or chemical properties. Since chemical characterization of a system in most cases is a whole different story than physical characterization, it requires different knowledge, technology and routine

analyses. Some properties of particulate systems are often related to the concentration of some component in the diluent or even concentration of the particles themselves. An example of this may be where adding additional salt or ionic solute destabilizes an electrostatically stabilized emulsion. The concentration dependence of particulate system stability will be beyond the scope of the present discussion, and therefore we will assume that the characteristics of a particulate system do not change when concentration changes.

Most physical properties of particulate systems are often ensembles or statistical values of the properties for their individual constituents. Commonly evaluated properties are:

- Concentration (number)
- Dimension (size)
- Shape (or conformation)
- Surface properties (area, charge, porosity, etc.)

The key needs for particle characterization arises when we encounter a system that is polydisperse with respect to the property in which we have interest. If all constituents of the system have the same property, there is hardly any need for an advanced characterization technology. All that is needed is a way to measure the macroscopic value. It is polydispersity that complicates the particle characterization process and makes it a branch of science. Polydispersity can refer to any property such as size, shape, material composition, mobility, color, etc.

4. Characterization of Particulate Systems

One focusing area in particle science and technology is the particle characterization in which particle concentration and size are of key interest. The behavior of a particulate system and many of its physical parameters are highly dependent on the size and number of particles present in the system.

Out of necessity, there are many techniques used in particle characterization, especially since the sizes, from nanometers to millimeters, and shapes, from solid spheres to porous flat plates, of particles needing to be characterized are extremely broad. Prior to modern particle characterization technologies, the only evaluation methods available were physical separation methods, such as sieving, which can only be used for particles larger than a few tens of micron. Additionally, these methods are usually based on the observation of certain macroscopic properties, those in most cases, consisting in averages of the constituents' properties and provide very little information about individual particles in the system. Optical microscopy may be the only exception to this, since it is a method that provides visual observation of individual particles with dimensions down to the micron range. However, the accumulation of adequate statistical data remains its weakness. This method was used in 1827 by the English botanist Robert Brown who discovered the random thermal motion of flower pollen particles in solution, now known as "Brownian motion." Today, many new sophisticated technologies are available that may be employed in particle characterization.

5. How We Define the Size of a Particle?

For a 3-D non-spherical or non-cubic particle, we will need more than one parameter to describe its dimension. However, the question is; can we choose just a few dimensional numbers to describe a 3-D particle? The answer is yes for objects with regular shapes such as a rectangle (2 or 3 dimensional numbers) or a cylinder (2 dimensional numbers). However, for irregularly shaped particles often encountered in the real world you will need a large amount of dimensional numbers to exactly describe the particle. If we are dealing with just a few particles, then it might be possible, although difficult, to obtain all the numbers necessary to characterize the dimensions of the particles. However, when talking about millions of particles, the ability to describe them individually is just not practical. Only one number (or maximum two numbers) should be used to "characterize" each particle. We call this number "size." This brings us to the conclusion that the definition we employ to define size will effect the answer we obtain. In fact, there are many different definitions for using a single number to describe a 3-D irregular particle. The most common one is to use an equivalent spherical representation, since a sphere is a 3-D object requiring only one number, the diameter, to describe it. If all the dimensional information of the particle is condensed into a single number, you must keep in mind that this single number will now contain distorted or summarized information about the particle, with the degree of distortion being related to the particle's shape. There are many different methods for converting 3-D irregular-shaped particles into equivalent spheres. The following definitions are just a few often appearing in the literature:

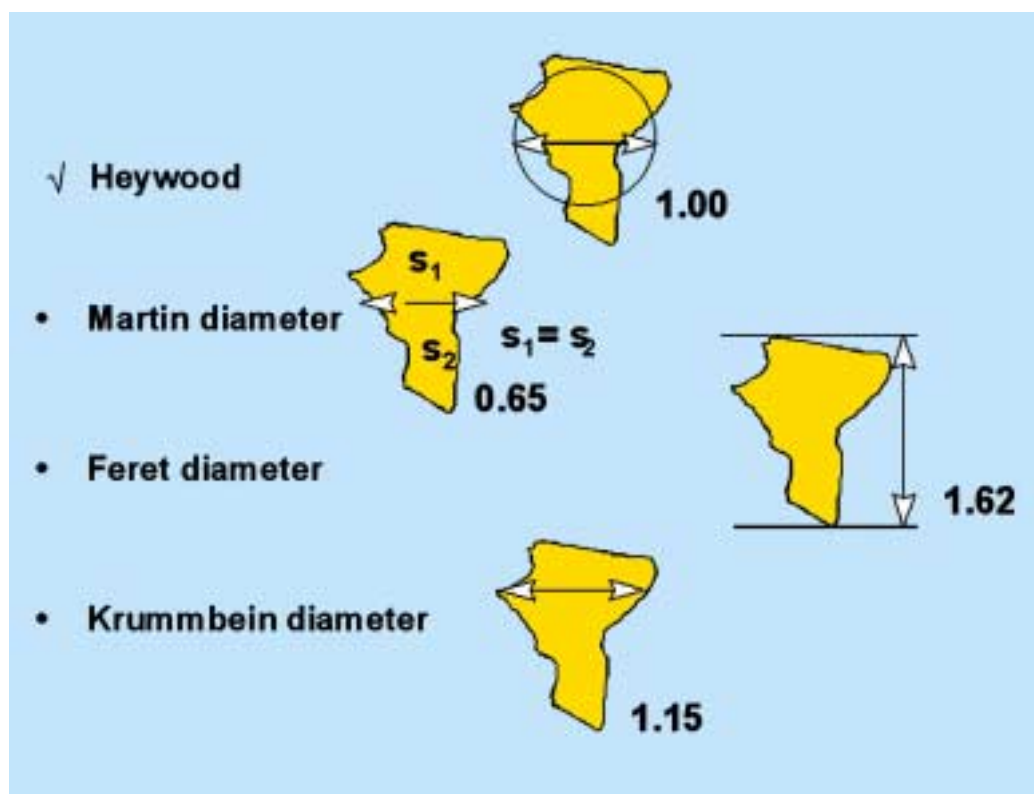


Figure 1.3 Different definitions of size

Sphere of same maximum length
 Sphere of same minimum length
 Sphere of same weight
 Sphere of same volume (Heywood diameter)
 Sphere of same surface area
 Sphere of same sieve aperture
 Sphere of same sedimentation rate

The volume equivalent sphere (the Heywood diameter) is the one used in most particle sizing technologies. However, the method we use to measure 3-D irregularly shaped particles will affect the results to be obtained in the form of equivalent spherical diameters. The spherical diameter distribution or the average diameter obtained using different technologies will have different bias and deviation from the true equivalent diameters of the particles because of both shape sensitivity of the technology and weighting effect for different particles in the sample. In a simple way, you are “seeing” a different view of the system that has been measured. One technique may see more of the large particles and another technique may see more of the small particles. Which result is right?

6. Which Technology Produces the Right Results?

Due to the nature of each technique, they will see the same system differently. In the language of statistics, different techniques see particles with different “weighting factors.” The mean value obtained from summarizing discrete individual values represents the relationship between the measured signal and the particle

It is very important to discuss what the differences are when using different technologies to obtain particle size. If we use an electron microscope to measure particles, we will measure the diameters, add them up and divide by the number of particles to get a mean result. If we then use laser diffraction to obtain the particle size we will see that the area of particles is what is most important and the size mean data will be generated from multiple diffraction patterns of many particles at the same time. In the Coulter Principle (Electric Sensing Zone) measurement, we would get the volume of each individual particle and then the result reported as the Heywood diameter. Let us assume that there is a particle system that consists of four spherical particles with the diameters being 1, 2, 3, and 10, respectively. Their corresponding mean values are shown in table 1.2 and figure 1.4.

Method	Mean Diameter (μ)
Electron Microscopy	4.00
Coulter Principle	6.37
Laser Diffraction	9.74

Table 1.2

We can see from the table above, that the mean value can be quite different if we use different technologies. The difference between number-averaged value and weight-averaged value resides on the fact that in the number average the mean value represents the values from particles with the largest population while in the weight average the mean value represents more from the particles with the largest

size. The same is true for the size distribution. The number distribution may be completely different from weight distribution.

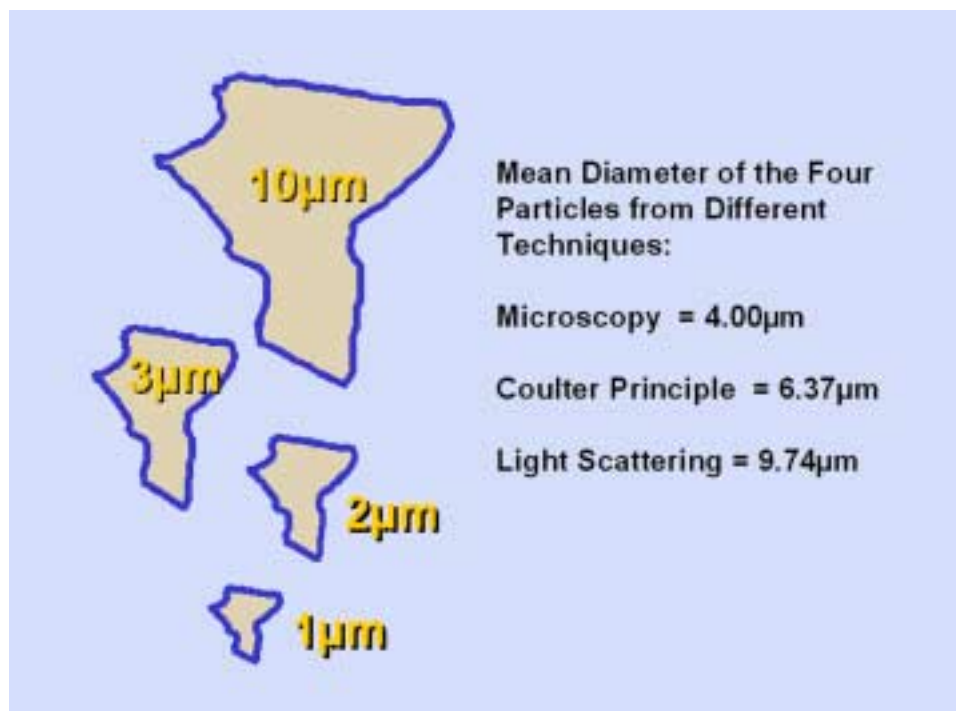


Figure 1.4

Because of resolution limitation or practical reason in actual measurement, the measured particle sizes are always classified into discrete channels (or bins). Each channel covers a range of particle sizes. For example, if an instrument has 100 channels in which Channels 10, 11, and 12 are marked 5µm, 10µm and 15µm, respectively, the particles classified into Channel 11 will have the diameters ranging from 7.5µm to 12.5 µm. Thus, even though the result may be presented as a continuous distribution curve, it really is a histogram with each channel centered at a nominal value with the high and low edges located in the middle of its nominal value and its neighbor channels' nominal value.

From the above analysis, we know that in order to compare the results, either the mean value or the distribution, obtained from different technologies, the results must be converted to the same ground, i.e., the same weighting. However, this conversion is based on an assumption that both technologies in comparison have the same sensitivity over the entire size range. Otherwise, the results will still be different even after conversion. For example, in laser diffraction measurements the scattered intensity from large particles will be buried into the experimental noise and undetectable if the measurement is performed at large scattering angles, while in Coulter Counter® measurements, the signals from small particles will be below the noise level if a large orifice is used. No matter what type of conversion is performed, the results from the two measurements will never be matched because there is different bias in the two technologies. One has to keep in mind that during the transformation the experimental error is also transformed. For example, if in the electron microscope measurement there is a $\pm 3\%$ error on the mean size, when the

number mean size is converted to a mass mean size the error will be cubed or become $\pm 27\%$! Of course, in the reversal transformation, a $\pm 3\%$ uncertainty in the mass mean size will be reduced to $\pm 1.5\%$ in the number mean size. Another often-used conversion is from mass % to volume % or vice versa. In this conversion if all particles have the same density then the two distributions will have the same shape, i.e. mass % = volume %.

7. Most Common Statistical Parameters Used in Particle Sizing.

In particle characterization, statistics can be calculated either arithmetically or geometrically. Arithmetic statistics has the conventional meaning as being used in other scientific disciplines. Geometric statistics is calculated based on the logarithm of the data. It is used when the data are arranged logarithmically, e.g., the logarithm of the size channels being linearly spaced, in the case of many particle-sizing instruments.

Mean It is an average of the whole distribution. There are different types of means, the most common types of means applied to size measurements are:

- ◆ geometric mean diameter
- ◆ diameter-weighted geometric mean diameter
- ◆ volume-weighted geometric mean diameter
- ◆ arithmetic mean diameter
- ◆ diameter-weighted mean diameter
- ◆ volume-weighted mean diameter
- ◆ mean surface diameter
- ◆ mean volume diameter

Median: It is the particle of such a size that it divides the population exactly into two equal halves. Note that populations with different weighting will have different values of median.

Mode: It is the most common value of the distribution, i.e., the highest point of the distribution curve.

Variance: A measure of distribution broadness defined as

$$V = \frac{\sum n(x - x_{\text{mean}})^2}{\sum n}$$

where n is the number of particles with diameter x.

SD: The square root of the variance is the standard deviation.

Skewness: The degree of distortion from symmetry of a distribution. When a distribution is perfectly symmetrical the skewness (g_1) equals zero. For right-skewed distribution the skewness is positive and for left-skewed distribution the skewness is negative.

$$g_1 = \frac{\sum n(x - x_{mean})^3}{(SD)^3 \sum n}$$

Kurtosis: A measure of the peakedness of a distribution using a normal distribution as the standard that has zero kurtosis (mesokurtic). When the distribution is narrower or sharper than the normal distribution, the kurtosis (g_2) is positive (leptokurtic). When the distribution is flatter than the normal distribution the kurtosis is negative (platykurtic).

$$g_2 = \frac{\sum n(x - x_{mean})^4}{(SD)^4 \sum n} - 3$$

Distribution: It is the continuous population frequency of the system. The shape of the distribution depends on each individual particulate system. There are distributions, which are commonly found in many industrial particulate systems, that can be manipulated in explicit mathematical forms.

The purpose of the statistical parameters is to provide information on particle-size distributions in a way suitable for relating them to physical or physiological processes or product properties. The contribution of statistics to the science of particulate systems seems insufficient. Probably, statistics is a science we are not always quite familiar with: Drawing conclusions about the properties of a population of particle sizes on the basis of properties of samples, requires statistical knowledge of relationships between sample and population properties.

The median is an statistical measure for locating a distribution, based only on a ranking of particles according to their sizes. From its definition it follows that e.g. the median value of a number density distribution will not change when some or more of the largest particles are given a ten-fold size. The median of a sample is not the best estimator for the central value of a lognormal size distribution.

8. Data Interpretation

Many times there is confusion with the interpretation of the statistical data of a size distribution. The values for the mean, median, mode, etc. in many cases will be totally different depending upon which distribution you are looking at (volume or number). The question arises; how this can happen? Which one is the right one? Both are correct, it all depends on the kind of data offers the most relevant information.

Suppose we have 200 bb's (ball bearings), 20 marbles and 2 golf balls. If they are counted, the total number is 222. This means that the bb's are 90% of the total population, the marbles 9% and the golf balls just 1%. But if we want to know the contribution in volume to the total, we have to measure their volume. In this case, the bb's contribute 25% to the total volume, the marbles 25% and the golf balls 50%. (Figure 1.5)

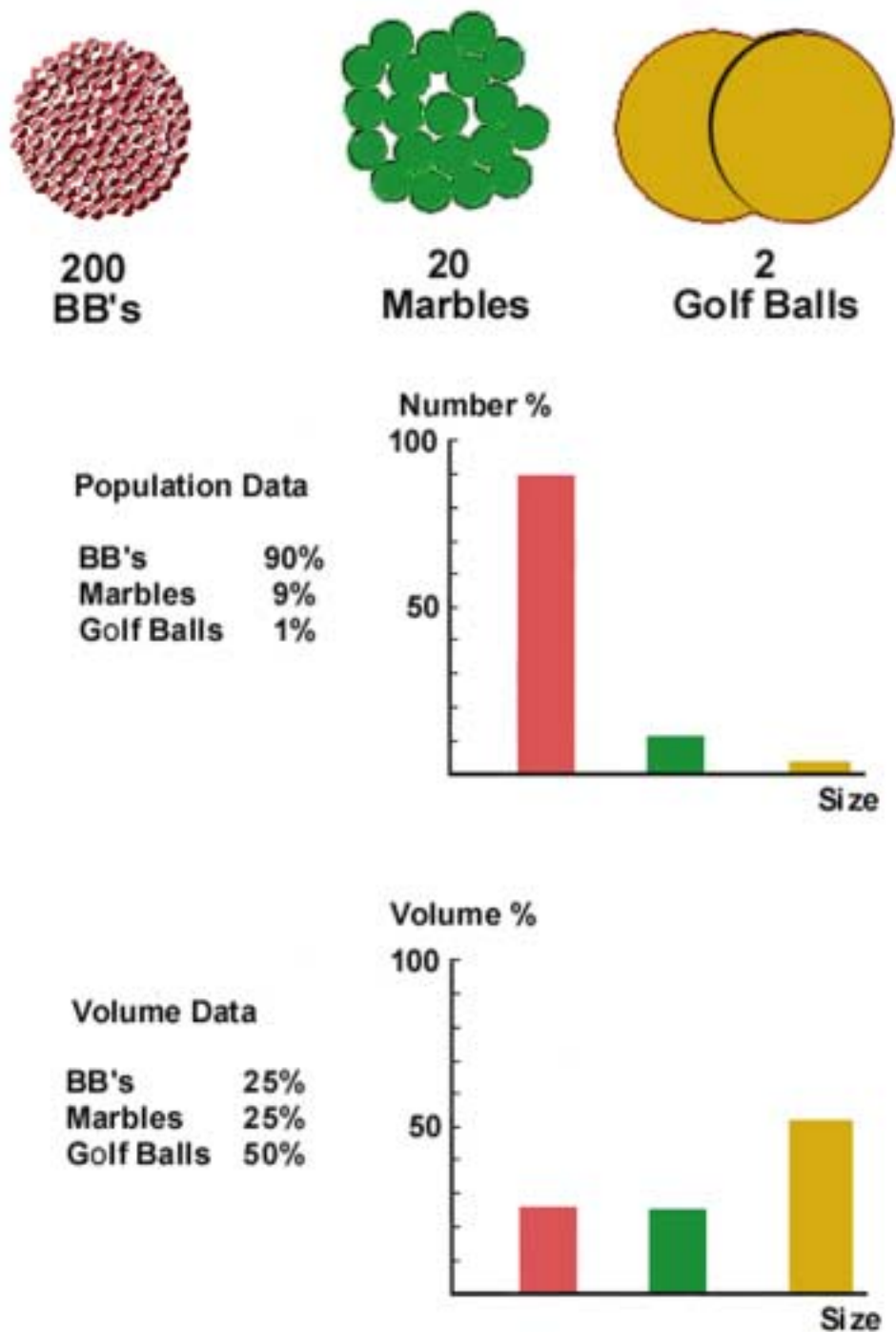


Figure 1.6 Number Size Distribution vs. Volume Size Distribution

When we look at a number size distribution graph, we are looking to the population of the particles. In general most powder grinds have more fines than large and because of this, the graph have a tendency to shift towards the lower size of the distribution. (Figure 1.6)

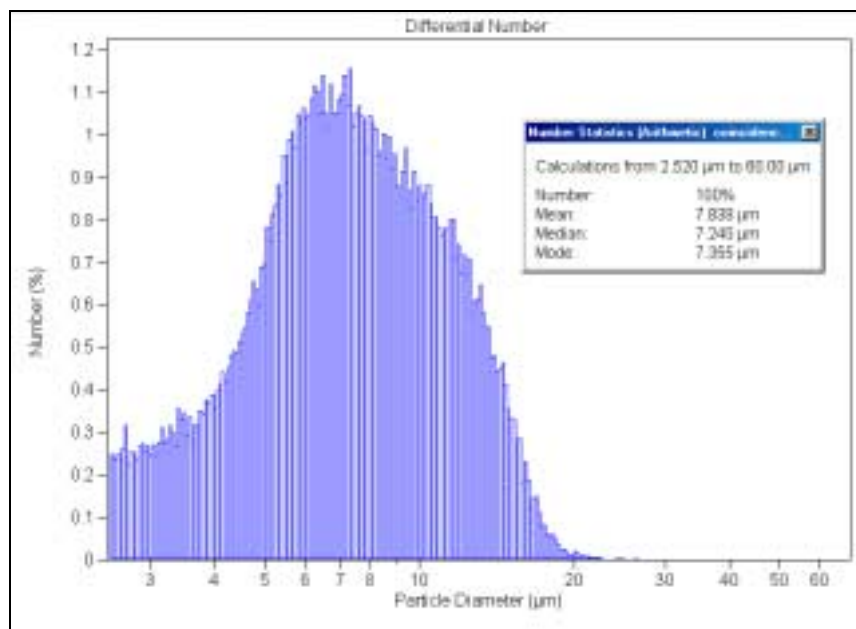


Figure 1.6 Number Size Distribution

In a volume size distribution graph, since the larger particles have the most volume displacement, the shaped curve will be shifted to the larger sizes. This graph is very similar to the results obtained from running your sample through a sieve set. (Figure 1.7)

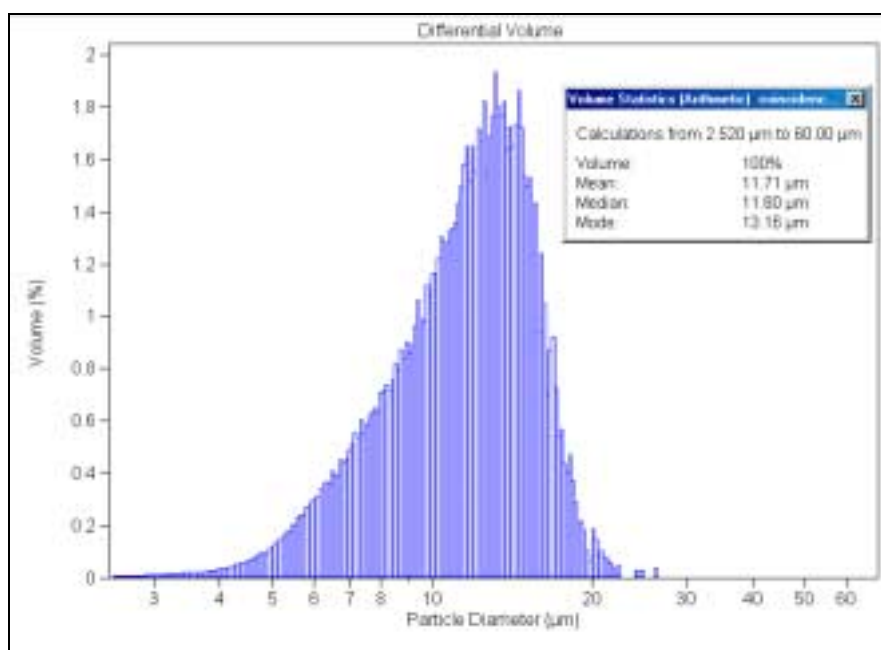


Figure 1.7 Volume Size Distribution

Chapter 2 THE COULTER PRINCIPLE

1. Background

While under contract to the United States Navy in the late 1940s, Wallace H. Coulter developed a method for counting and sizing particles. The method was principally developed to count blood cells accurately and quickly. Its acceptance in the field of hematology is evident in that presently over 98% of automated cell counters incorporate the Coulter Principle. In the past fifty years, the method has also been also utilized to characterize thousands of different biological and industrial materials. Bacteria, yeast cells, drugs, pigments, toners, foods, abrasives, explosives, clay, minerals, metals and many others have all been analyzed by the Coulter Principle. The method may be used to measure any particulate material that can be suspended in an electrolyte. Particles as small as 0.4 μm and as large as 1200 μm in diameter can be analyzed. The method is described in the International Standard ISO 13319 and is the subject of several ASTM standards. Over 7000 references to the uses of various COULTER COUNTER® models have been documented.

2. The Coulter Principle (Electrical Sensing Zone)

2.1 How Particles are Sized and Counted

If an aperture is placed between two electrodes and a current path is provided by a low concentration electrolyte, a resistance can be measured between the electrodes. The aperture creates what is called a “sensing zone.” Particles in low concentration, suspended in the electrolyte, can be counted by passing them through the aperture. As a particle passes through the aperture, (Figure 2.1), a volume of electrolyte equivalent to the immersed volume of the particle is displaced from the sensing zone. This causes a short-term change in the impedance across the aperture. This resistance change can be measured as a voltage pulse or a current pulse. The voltage pulse is proportional to the volume of the sensed particle

Using counter and pulse analyzer circuits, the number and volume of particles passing through the sensing zone can be measured. The volume may be represented as the equivalent spherical diameter. The measured particle sizes can be channelized using a height analyzer circuit and a particle size distribution obtained. The electrical response of the instrument is essentially independent of the shape of particles with the same volume, an exception to this may occur with some extreme shapes. Color or refractive index of the particles does not affect the results.

Simple COULTER COUNTER systems may have only one counter and size level circuit, while more complex systems can obtain particle size distributions automatically in up to 300 size channels. These measurements are made in only a few seconds as counting and sizing rates of up to 10,000 particles per second are possible. The accuracy of these size measurements can be better than 98%. To count the number of particles in a known volume of suspension, such as for particulate contamination studies or a blood cell count, the sample volume must be exactly known. Originally, a simple mercury manometer was used. A mercury-free

piston displacement metering system is now in use in recent models of COULTER COUNTER.

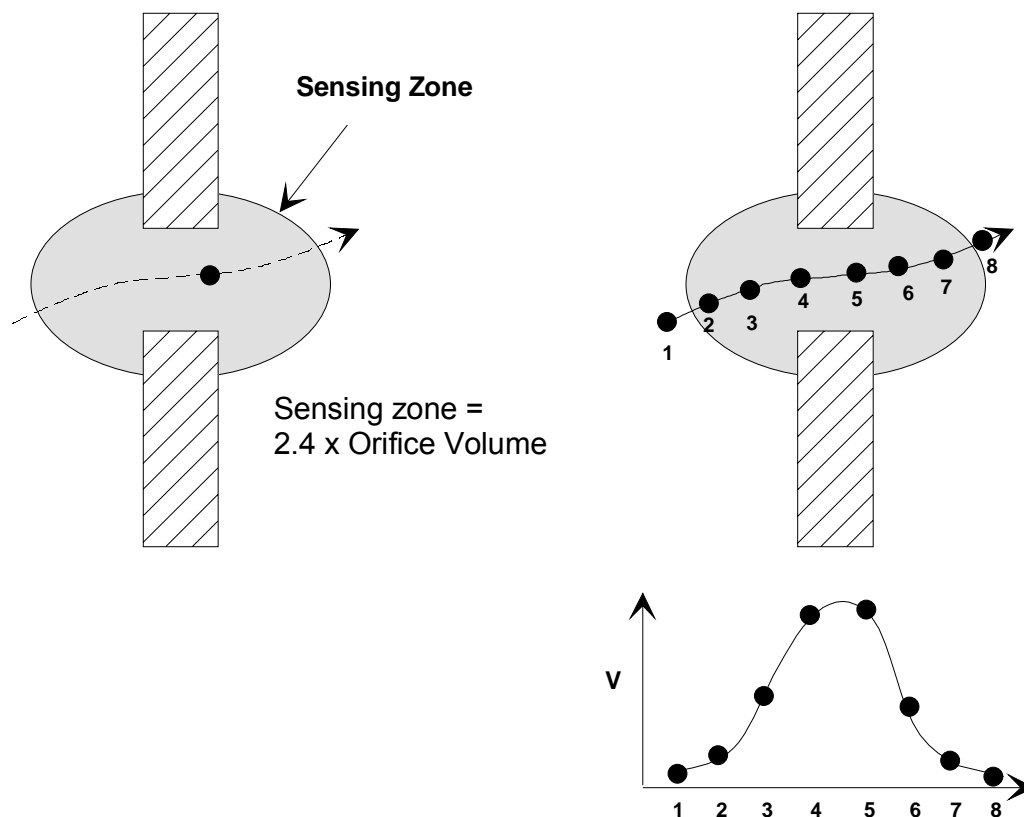


Figure 1.2 Pulse generation

The Multisizer™ 3 is the latest generation of COULTER COUNTER, (Figure 2.2). It uses a Digital Pulse Processor (DPP) for high-speed digitalization of the signal allowing the use of pulse area analysis and other techniques for additional particle characterization. The data no longer has to be processed and compressed on the fly but can be stored without loss of information. This enables the raw data to be reprocessed using different settings and to show sample changes over the length of the run.

A peak begins when the signal rises above the noise threshold and ends when the signal falls below the noise threshold. The signal is scanned several million times per second and then the following information is extracted:

- ◆ max height
- ◆ peak width
- ◆ mid height
- ◆ max height gain stage
- ◆ mid height gain stage
- ◆ peak area

Together these parameters give a much better indication of the shape of the pulse than was available in the past and together make it possible to obtain a more accurate particle count and size distribution.

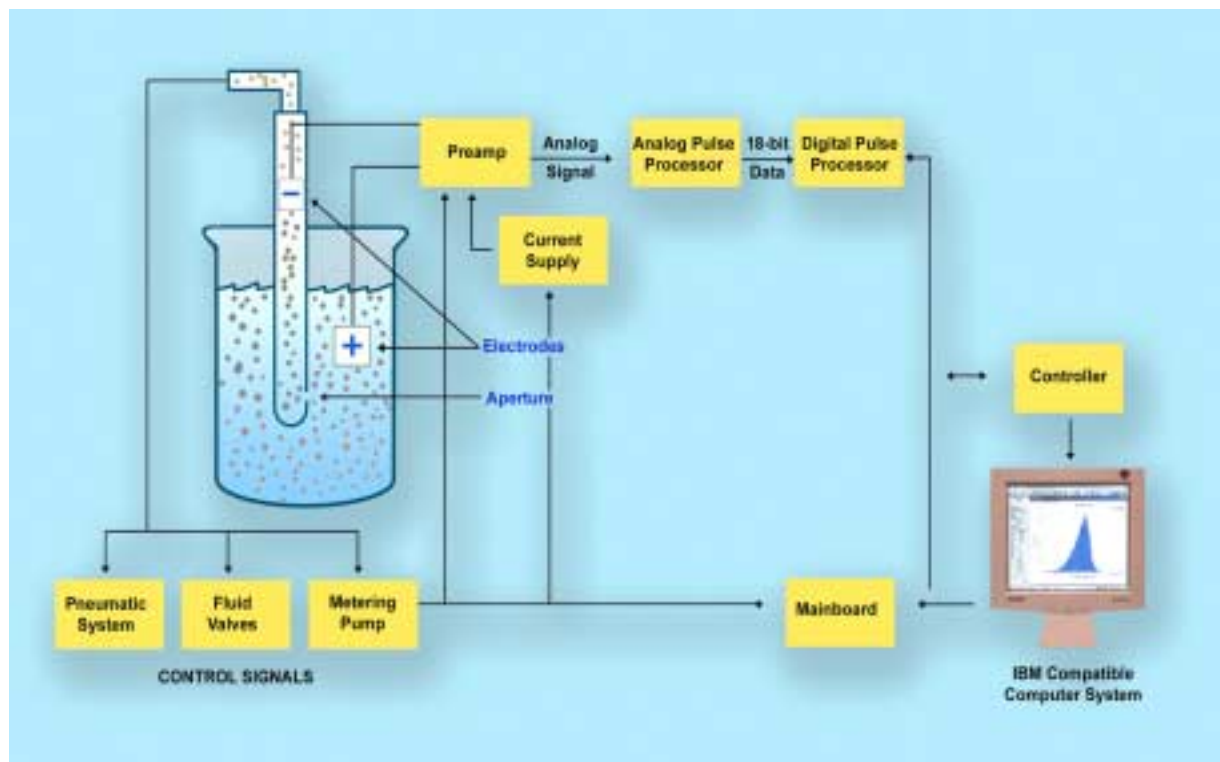


Figure 2.2. The Multisizer™ 3 Analyzer Schematic

2.2. Coincidence

The concentration of the particles to be measured should be sufficiently low such that they can be counted one at a time. However, depending on the volume of the sensing zone and to a lesser degree the response time of the electronics, two or more particles will be in the sensing zone at the same time. This gives rise to only one measured signal. This effect is called coincident particle passage. There are two effects in coincident events. Primary coincidence is as described above where two particles are counted as one of a larger size (Fig. 2.3), it results in lower particle counts, but also it has an effect on the size distribution. If the percentage of coincidence correction is high, the size distribution can be shifted. Secondary coincidence leads to an error in size distribution. This occurs when two particles, both smaller than individually detectable, are registered as one larger particle (Fig 2.4). In Figures 2.5 and 2.6, the same sample was run under the same conditions but at different concentrations $A > B > C > D$. The effect of coincidence on size distribution is only noticeable when the coincidence correction is above 30-40%.

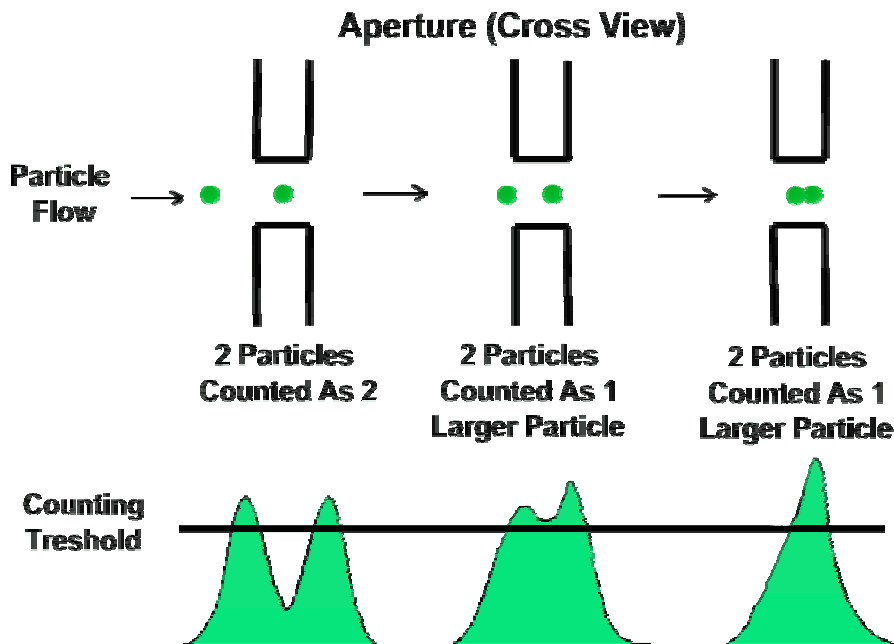


Figure 2.3 Effect of Primary Coincidence

Primary coincidence up to 10% (where one particle in 10 is not being counted) is easily corrected mathematically, this correction is automatically performed by the Multisizer 3 software. Primary coincidence correction is based upon the number of particles counted in the metered volume, the diameter of the aperture, and the empirical data related to the effective volume of the sensing zone.

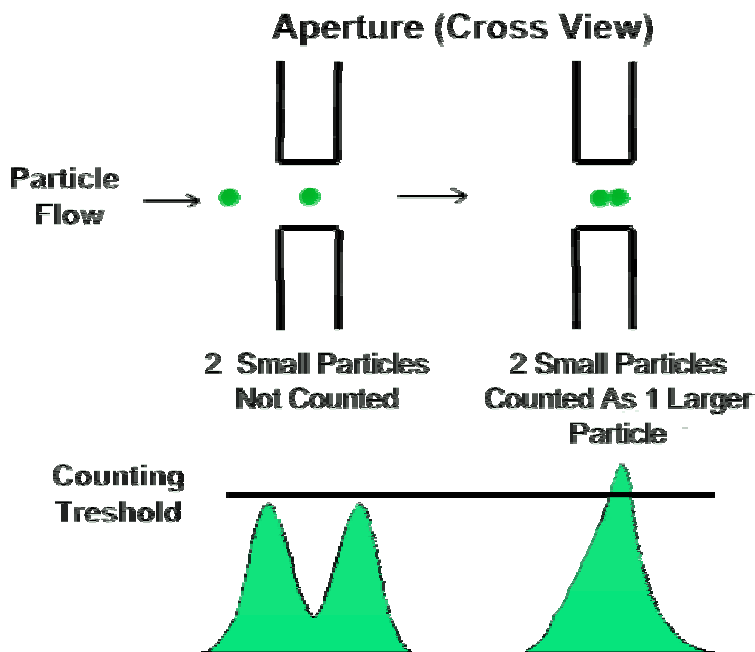


Figure 2.4. Effect of Secondary Coincidence

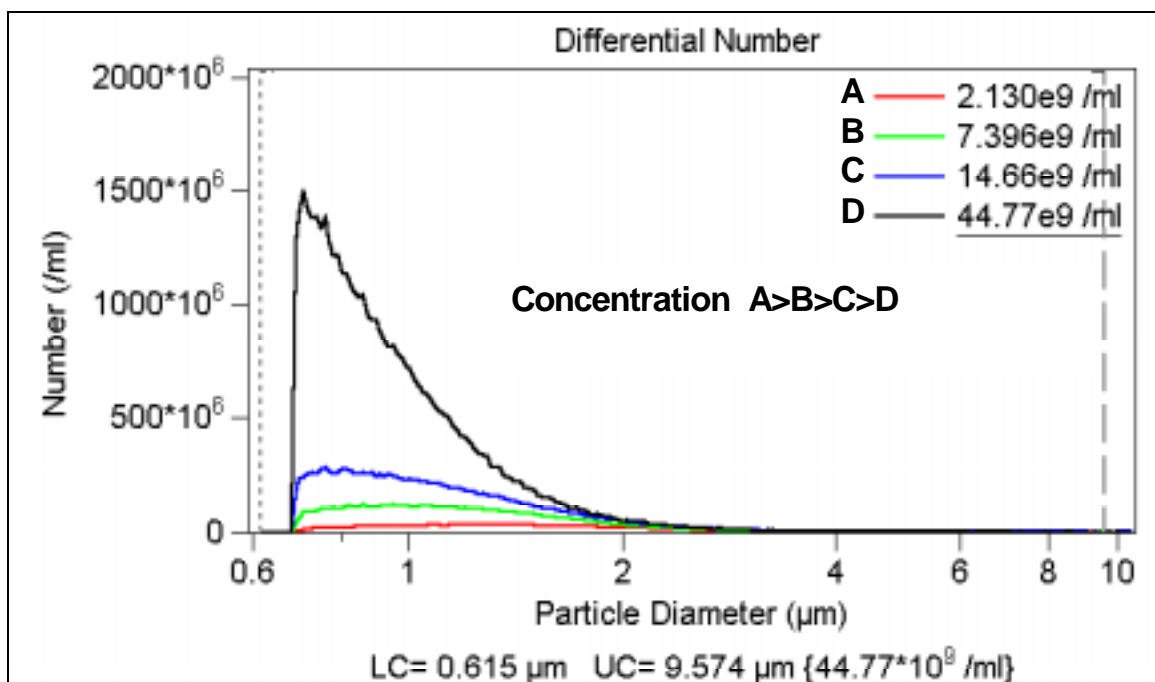


Figure 2.5 Effect of Coincidence Correction on Counts

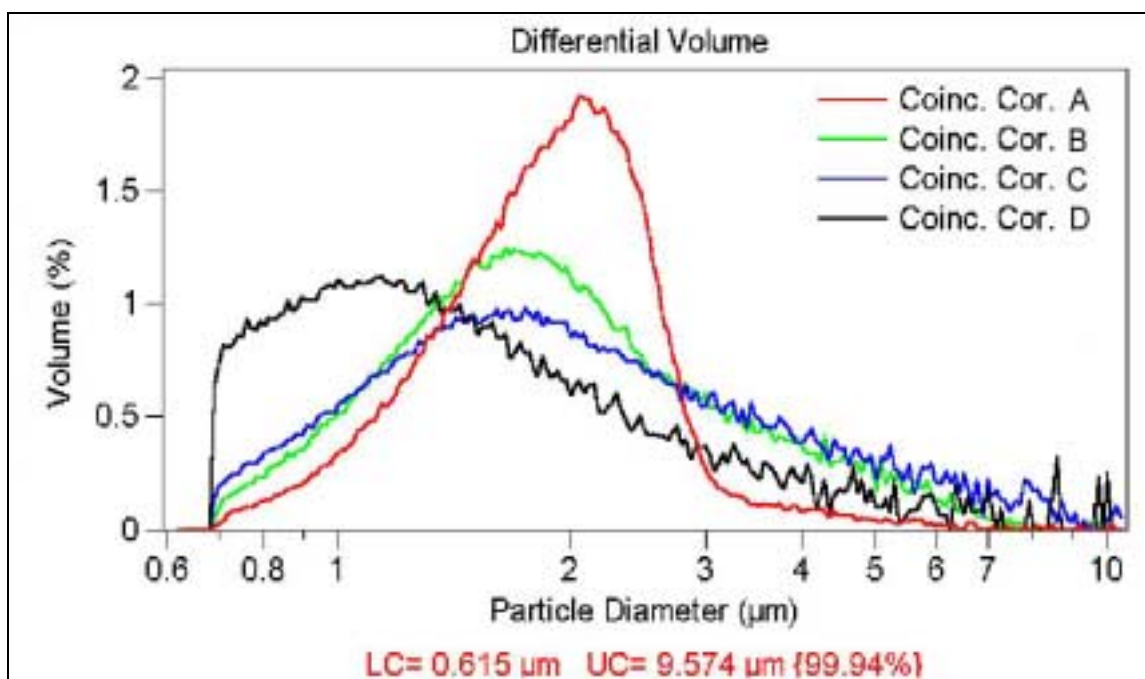


Figure 2.6 Effect of Coincidence Correction on the Size Distribution

2.3. Counting and Coincidence Correction

At the noise threshold the instantaneous concentration is measured for use by the concentration meter. This gives the user a means of checking the concentration of the sample.

At the count threshold the number of pulses are counted using the same criterion as used for the peak data. Thus the count should be equal to the number of pulses stored in the peak buffer if the threshold was set equal to the count level. The mark and space counts at the count level are accumulated for the whole run. This information is used to coincidence correct the size distribution. Typically the count threshold would be set to the left hand edge of the size distribution at the start of the run. The noise threshold can then be set at a lower level to allow collection of peak data below the count level without reducing the accuracy of the coincidence correction above the count threshold.

2.4. Particle Path Through the Aperture. Editing Function

When particles are drawn through the aperture they come from all points around the aperture. In other words some particles will pass directly through the center of the aperture, while other particles will pass through nearer to the edge of the aperture. All particles passing through the aperture will displace their volume of electrolyte solution and generate a voltage peak. However, those particles that do not pass through the center of the aperture will produce a slightly larger peak than would be expected for their displaced volume. This slightly larger “artifact” pulse causes the particle size distribution to be skewed very slightly to the larger sizes (Figure 2.6).

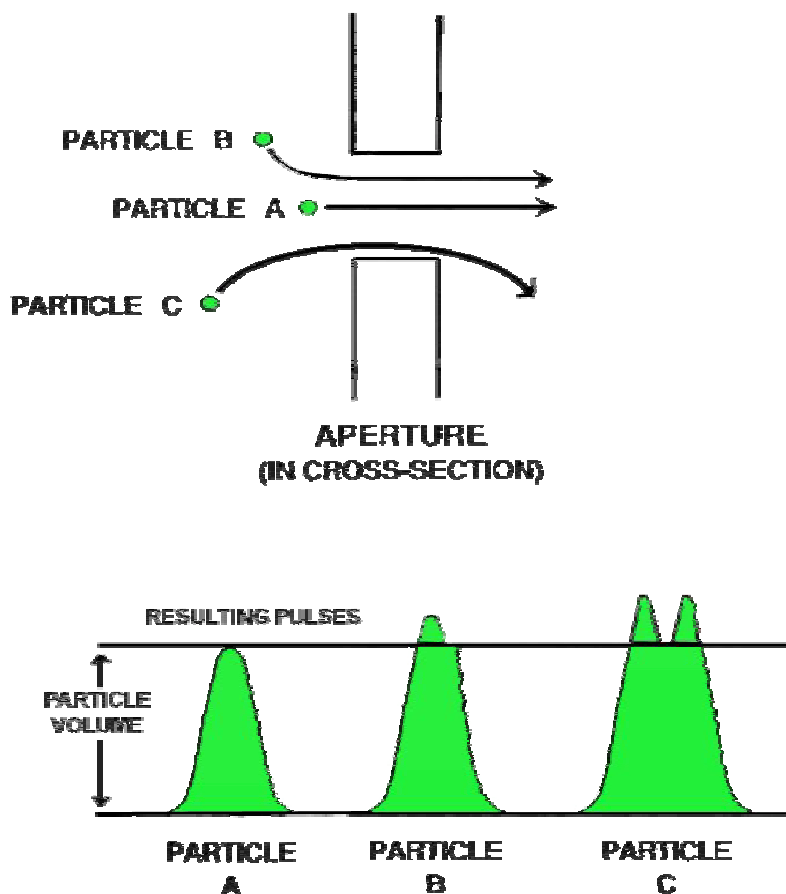


Figure 2.6. Pulse Shape Depends on the Particle Flows Through the Aperture

The fluid velocity and, hence, the particle velocity, also play a role in the distribution of the particle size. This is due to the fact that at the aperture walls the velocity is lower, therefore, producing pulses that are wider and higher in amplitude. This causes the size distribution to be skewed towards the larger sizes (Figure 2.7). These effects are most significant and noticeable on very narrow size distribution materials such that in extremely narrow distribution materials the artifact peak can manifest itself as what appears to be a second size population. On wider size distribution materials such as most powders and suspensions, this effect is neither significant nor observable.

Many of these artifact peaks can be electronically screened by examining each pulse and “editing” the unwanted ones out of the analysis data. The use of the Edit facility removes the distorted pulses, so that the recovered size distribution is more nearly correct (Fig 2.8) Typically, up to 50% of the particle-generated pulses are discarded in order to enhance the sizing accuracy.

To gain most benefit from the Edit facility:

- ◆ The aperture tube used should be in the range 15 μ m to 280 μ m diameter.
- ◆ The particle size distribution to be measured should be uni-modal, not exceeding approximately 2:1 by diameter in overall range and lie at a modal diameter which is less than approximately 15% of the aperture diameter to be used.
- ◆ The concentration of the particles should be less than approximately 10% aperture coincidence level.

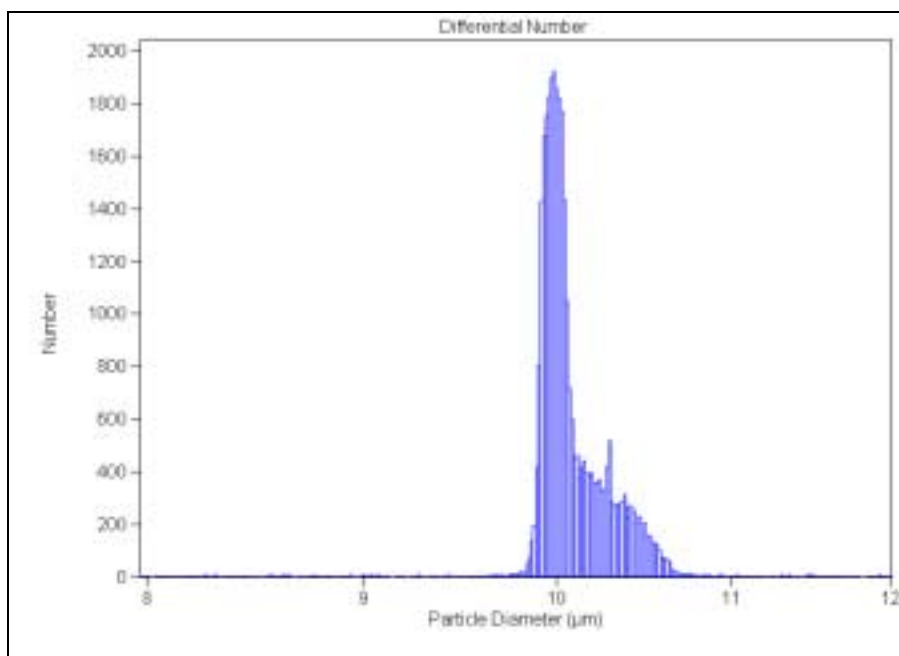


Figure 2.7 Size distribution not using the Edit function

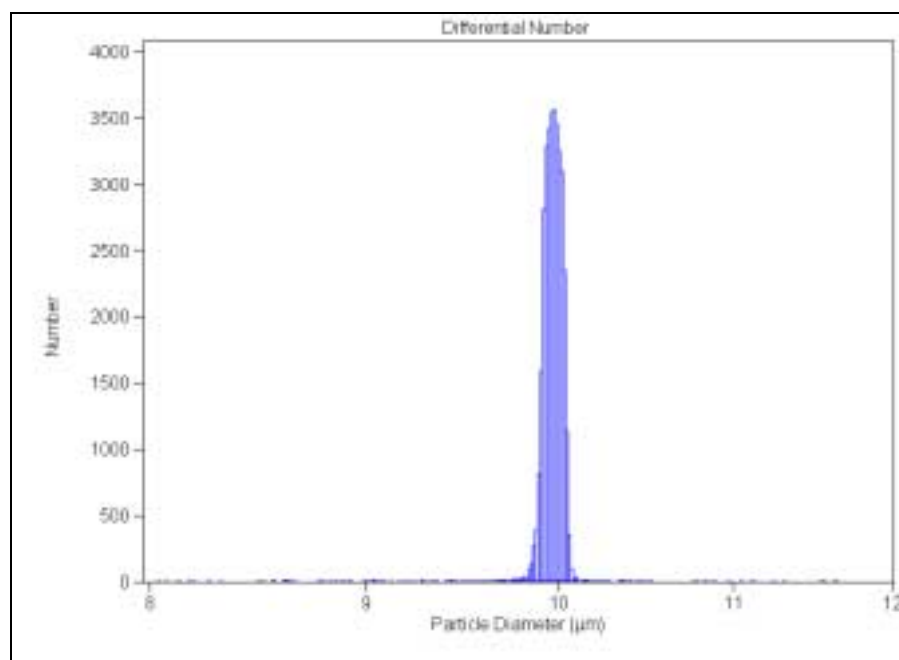


Figure 2.8 Size distribution using the Edit function

2.5. Enhanced Editing

The use of a longer tunnel aperture may improve the accuracy of the results for samples with a narrow size distribution. The behavior of long tunnel apertures is mostly due to the greater approach to parabolic flow inside the bore (Fig. 2.9). True parabolic (streamline) flow is never reached in any usable aperture, because pipes need to be at least 100 times their diameter for this type of flow to develop properly.

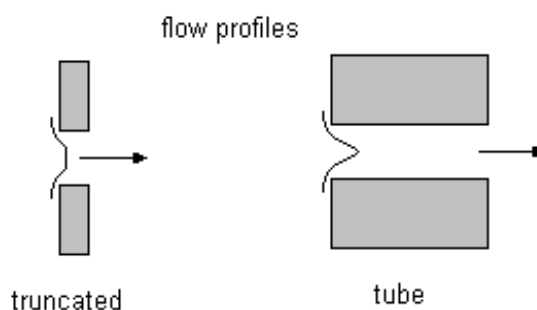


Figure 2.9. Cross-section of an standard aperture and a long tunnel aperture.

When liquid flows in streamline flow in a tunnel, the bulk of the liquid can be shown to flow along a periphery at $0.7R$ from the axis where R is the radius of the tunnel. The fastest streamline is at the center, (Fig. 2.10). Hydrodynamic forces tend to move particles towards the fastest. This is because for real particles the velocity across their diameters varies. Particles try to embed themselves in the average flow velocity and the average velocity is always half the peak or central velocity. Long tunnels allow more particles to be embedded in this flow than ordinary short apertures do. However, because of the larger “volume” of such apertures, these apertures have a

more restricted dynamic range and appear noisier at 2% of their diameter than standard tubes offsets this. But strictly speaking, the 2% level is a higher size because the aperture “volume” is larger.

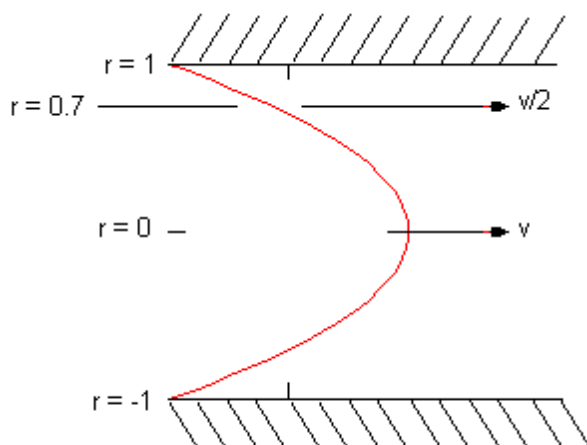


Figure 2.10. Velocity profile in parabolic flow

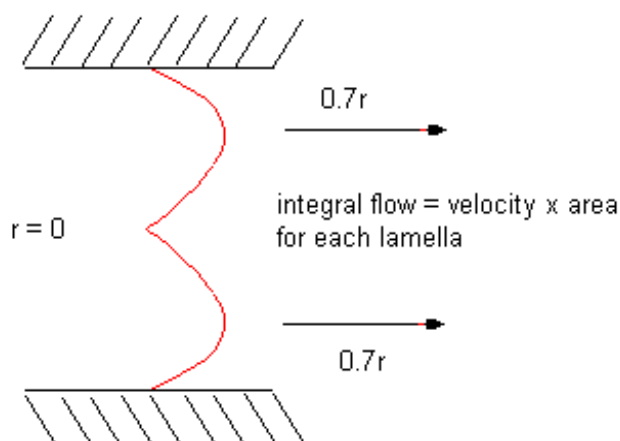


Figure 2.11. Bulk flow profile for parabolic flow in a tube with circular cross-section. Most particles travel along the line marked $0.7r$

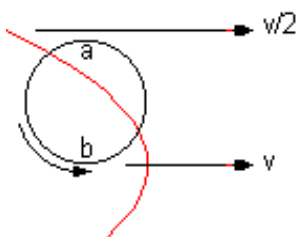


Figure 2.12. Hydrodynamic forces on a particle in parabolic flow.

The flow in a cylindrical tunnel can be imagined as a series of concentric “cylinders” each travelling at a velocity that follows the parabolic profile in figure 2.10. The bulk fluid flow will be the area of each “cylinder” multiplied by its velocity. The result has the profile shown in figure 2.11. Particles are embedded randomly at essentially a uniform concentration in all the cylinders but the number of particles per second

recorded by a counter will be greatest in the cylinders with the highest bulk flow. So, the particle flow rate has the same profile as the fluid bulk flow, i.e. as in figure 2.11.

In figure 2.12, $v > v_2$, because point **a** on the particle is travelling at $v/2$ and point **b** is travelling twice as fast, there must be a rotational force on the particle. Also, there must be a net force roughly tangential to the parabola tending to move the particle towards the faster streamline at v .

Figure 2.11 shows the bulk flow of liquid at 0.7 radius, the majority of particles travel at this streamline. In a short tunnel, particles close to the wall have too little time to move towards 0.7r, whereas in a longer tunnel the opportunity to do so increases. Consequently, longer tunnels give a greater proportion of pulses in the main peak. Pulses from 0.7r to 0.0r have essentially “bell” shaped pulses whereas those from the wall have “M” shapes resulting from the particles crossing regions of high current density at both the entrance and exit of the aperture.

Edit circuits work on the width W of pulses relative to their heights H . Longer tunnels stretch pulse widths out and because parabolic flow is more established stretches pulses from the wall of the aperture much more than pulses from the 0.7r streamline.

Longer tunnels also decrease pulse heights, which means that the ratio W/H goes as the square of the tunnel length change. All this means that edit circuits, have a much better opportunity to discriminate pulses (Fig. 2.13).

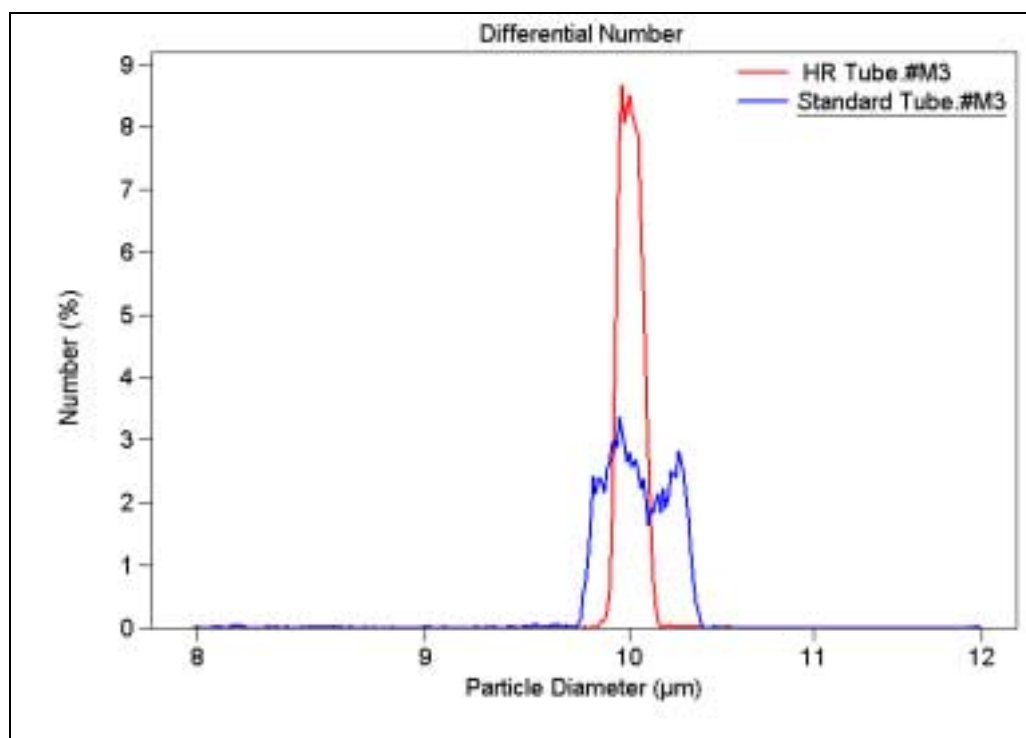


Figure 2.13. Effect of using a high resolution aperture tube compared to an standard aperture tube

Aperture tubes with a longer tunnel for using in the Multisizer 3 are manufactured in 50 μ m, 70 μ m, 100 μ m, 140 μ m and 200 μ m sizes; they are called High Resolution Tubes.

2.6. Particle Sizing Response

For an aperture of length-to-diameter ratio of approximately 1:1 or 2:1, the electrical signal depend on both particle and aperture parameters.

$$vR = \frac{i}{I}$$

where v = particle volume
 I = aperture current
 i = pulse intensity
 R = a response factor relative pulse height per unit volume V

$$R = \frac{i/I}{v}$$

$$V = \frac{v}{i/I}$$

$$v = V(i/I)$$

$$V = \frac{1}{R}$$

$$V = 0.4852U^2(L + 0.93U)$$

where

$$U = D^{1.0556}$$

D = aperture diameter

L = aperture length

The voltage response is guaranteed to be linear between 2% and 60% of the aperture diameter. Experiments have shown linearity response up to 80% of the aperture diameter, however up to 60% is guaranteed. Below 2% of the aperture diameter the noise dramatically increases.

2.7. Calibration

The Coulter principle provides two basic measurements, particle count and particle volume. The particle count requires no calibration. The principle yields particle counts that can be regarded as accurate, subject to coincidence correction. However, the particle size response must be calibrated.

The ideal method of calibration is the mass integration, sometimes called the mass balance. The method is dependent upon using calibration materials that are of uniform density. The particles to be measured are used to calibrate the instrument using standard gravimetric and volumetric methods. The K_d (diameter calibration factor) can be calculated from

$$K_d = \left[\frac{6WV_m 10^{12}}{\pi V_T \rho (\Delta n V)} \right]^{1/3}$$

where

W = mass of sample in beaker (g)

V_T = volume of electrolyte solution in which W is diluted (mL)

V_m = manometer volume (mL)

ρ = immersed density of the particles (g/mL)

Δn = number of particles in the size interval

V = arithmetic mean volume for that particular size interval (e.g. product of threshold value, aperture current, and attenuation)

Not all particulate materials are homogeneous in terms of density. For this reason, and for general convenience, it is more common to perform a calibration using standard materials, such as a polymer latex sample. Calibration standards can be obtained from Beckman Coulter Inc whose Quality System is certified to ISO 9002 and NIST traceable. These are narrow-size-ranged latex samples whose size has been precisely measured by another method and they are standardized for the mode value. The material is measured and the mode of the generated size distribution is related to the assayed value of that latex.

$$K_d = \frac{J \cdot \sqrt[3]{I \cdot G \cdot d}}{X}$$

where

J = number of channels selected (64, 128 or 256)

I = current μA)

G = gain

d = calibrator size (μm)

X = modal channel, left edge (μm)

Regardless of the method of calibration, the calibration will be only valid for the entire range of size measurements from an aperture/electrolyte combination. Calibration drift is not common, but good laboratory practice dictates that routine calibration verification control tests be performed.

The Multisizer 3 provides automatic calibration and verification. This feature ensures that instrument calibration is uniform independently of operators, shifts or different locations.

2.6 Apertures

2.6.1. Aperture Diameter

Aperture diameter for standard tubes ranges from 20 μm to 2000 μm . Each aperture can be used to measure particles within a size range of 2% to 60% of its nominal diameter (Table 2.1). Therefore, the overall particle size range of 0.4 μm to 1200 μm is possible. The ability of the method to analyze particles is limited to those particles that can be suitably suspended in an electrolyte solution. The upper limit therefore

may be 500 μm for sand but only 75 μm for tungsten carbide particles. Apertures smaller than 15 μm have been produced. However, the lower size limit is restricted by electronic noise generated mainly within the aperture itself. The following table lists the measurable particle size ranges for standard aperture diameters.

μm	Aperture diameter, μm
0.4 - 12	20
0.6 - 18	30
1.0 - 30	50
1.4 - 42	70
2.0 - 60	100
2.8 - 84	140
4.0 - 120	200
5.6 - 168	280
8.0 - 240	400
11.2 - 336	560
20.0 - 600	1000 *
40.0 - 1200	2000 *

* The maximum size range with 1000 μm and 2000 μm aperture tubes is dependent upon the density of the material being analyzed.

Table 2.1. Particle Size Ranges with Standard Tubes

2.6.2. Analysis Ranges with Standard Tubes

Selecting the most suitable aperture size is dependent upon the particles to be measured. If the sample to be measured should be composed of particles largely within a 30:1 diameter size range, then the most suitable aperture can be chosen. For example, a 30 μm aperture can measure particles from about 0.6 to 18 μm in diameter, a 140 μm aperture can measure particles from about 2.8 to 84 μm .

If the particles to be measured cover a wider range than a single aperture can measure, two or more apertures have to be used and the test results overlapped to provide a complete particle size distribution analysis.

Another consideration regarding smaller apertures is that they are more prone to occlusion due to debris in the sample suspension or agglomeration of the sample itself. With the Multisizer II and other Coulter instruments, the aperture is monitored optically so that any blockage to the aperture is visually evident. An oscilloscope also displays the voltage pulses making aperture blockages more apparent. Blockages are normally very easily cleared by means of momentary backpressure to the aperture or by using a soft brush. Instruments for blood cell counting incorporate a "burn" circuit. By applying high current to the aperture, the occlusion can thus be removed. In the case where the above means do not remove the blockage, the aperture tube can be removed from the system and cleared by means of soaking in acid or application of greater backpressure. When using small apertures, special care has to be taken with high current because it can damage the aperture. For larger

aperture tubes, careful and extremely brief application of low power ultrasonic vibration can be used.

2.7. Electrolytes

Several considerations come into play when selecting the electrolyte in which the particles are to be suspended. The solution should be chemically compatible with the sample material and should allow for proper sample dispersion. Frequently addition of surfactants and ultrasonication are necessary. See Chapter 5 for a more complete description of sample dispersion.

The solution must also be essentially free of particles within the range of particles to be measured. The electrolyte solution is commonly filtered using 0.45 μm or 0.22 μm membrane filters.

Electrically the solution should have roughly the same characteristics as 0.2-20% w/v sodium chloride in water. The aperture resistance measured in the solution should be between 1 and 100 k Ω , ideally around 5-40 k Ω . As the Coulter Principle was initially used for counting blood cells, the electrolyte most often used is normal Saline (0.9 g NaCl / 100 ml H₂O). A 5% trisodium orthophosphate solution is also commonly used, particularly in instances of measuring clays, silicas, or other minerals where chloride ions would inhibit dispersion. A lists of common electrolyte solutions used in COULTER COUNTER applications can be found in the Appendix section of this book.

As has been stated before, the Coulter Principle can be used to count and size any particles that can be suspended in an electrolyte. In order to suspend some large particles, it may be necessary to add a thickening agent such as glycerol or sucrose to raise the viscosity of the solution. A thickening agent will also help reduce the noise generated by the turbulent flow of low viscosity electrolyte as they pass through large diameter apertures ($d \geq 560 \mu\text{m}$). Even with maximum stirring and with thickening agents, large particles may not stay in suspension uniformly enough to enable a representative sample to be counted with reproducible results. It is because of the issue of particle suspension that round-bottom beakers have virtually replaced flat-bottom beakers as the vessel of choice with the Coulter Principle. A round-bottom beaker allows for a more uniform dispersion of particles throughout the suspension resulting in more consistent measurements.

2.8. Conductive Particles and Porous Particles

Conductive Particles. In theory, only some difference in conductivity is needed to generate a pulse but in practice, the discharge potential from conductor to ions (the reversible e.m.f.) creates a barrier that makes any conductor into a non-conductor provided the potential across the particle does not exceed the discharge potential. The voltage necessary to break the potential barrier is known as “breakdown voltage”.

Particles of conductive materials can be accurately measured by the electrical sensing zone method provided that the voltage applied across the aperture does not exceeded the breakdown voltage. To analyze a conductive material an initial test should be performed in order to get the optimum current that may be applied below

the breakdown voltage (Fig. 2.14 and Table 2.2). For the first run the current and gain are automatically set up by the instrument. Then a series of runs are performed decreasing the current in each consecutive run. The mean size will increase until reaching a point where it won't keep increasing. This point is when the applied current is below the breakdown voltage.

Particles of highly conductive materials which do not easily form surface layers such as copper, silver, and platinum can be sized correctly provided a very low voltage is used and the barrier increased, e.g. by adding a 0.5% solution of Cetrimide.

In the case of cells enclosed by membranes, the linear response to cell volume changes at some "breakdown" value of applied voltage. This value has to be taken into account when setting conditions for the analysis. Blood cell analyzers are designed to operate below the blood cells breakdown potential.

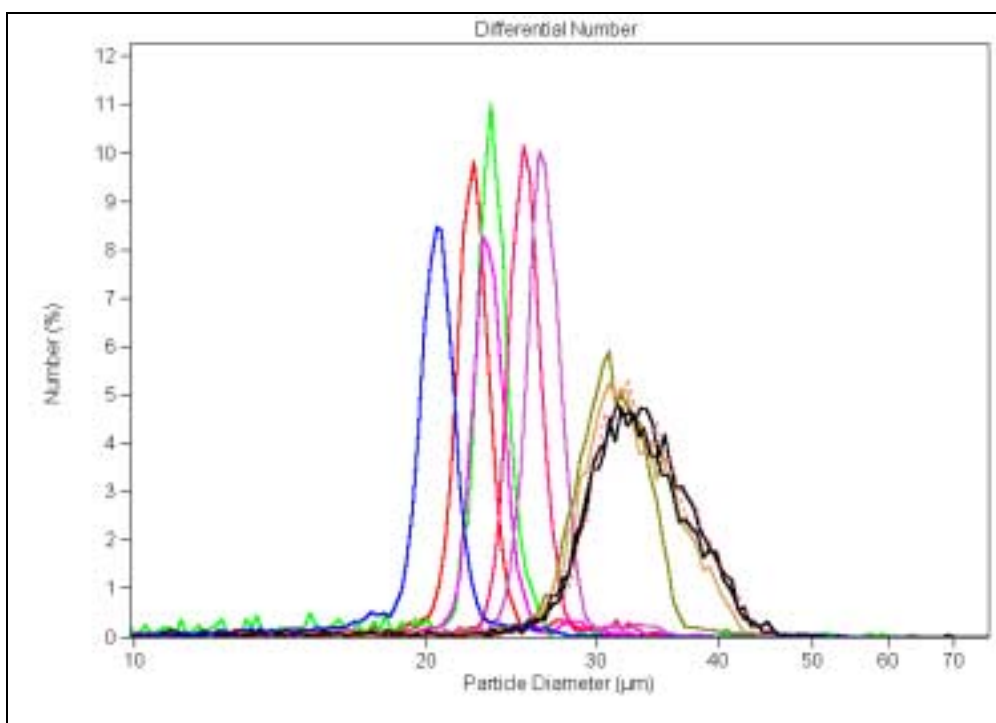


Figure 2.14. Determining the breakdown voltage

Mean (μm)		Mean (μm)	
Test 1	20.3	Test7	30.7
Test2	22.2	Test8	32.2
Test3	22.7	Test9	32.9
Test4	23.1	Test10	33.3
Test5	25.2	Test11	33.4
Test6	26.3	Test12	33.4

Table 2.2. Determining the breakdown voltage

Porous Particles. When a particle contains pores, the influence of particle porosity should be determined experimentally. For porous particles the volume of pores that are parallel to the electric field lines is not measured, while the volume of pores perpendicular to these lines does contribute to the measured volume.

In practice, however, the situation is far more complicated. The directions of the pores in a particle are randomly distributed. Pores may be interconnected, may pass through the whole particle or not, and may vary in size from less than 1 nm, up to several μm which means that not all pores may be wetted by the electrolyte. The only way to determine the influence of porosity is to measure a particle twice, first with open pores and then with all pores filled or closed with an inert medium. When the porosity of a particle is known, its influence on the measured size may readily be established.

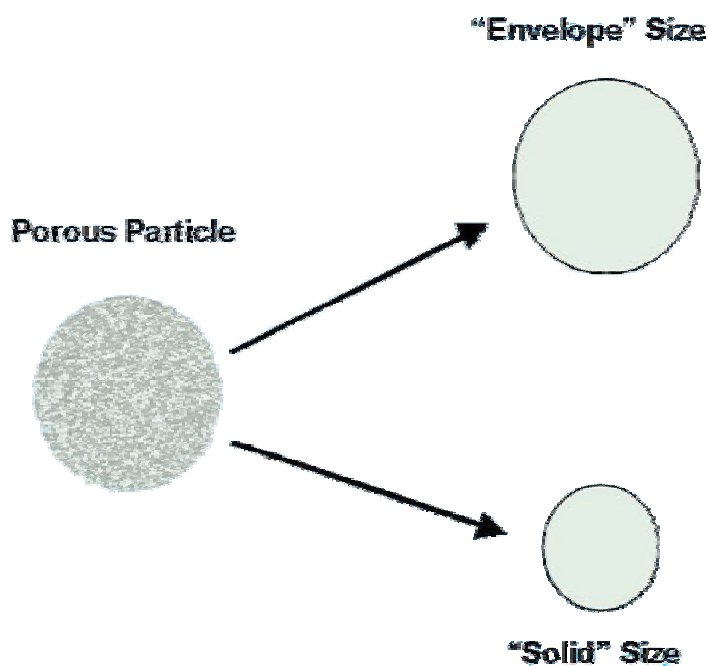


Figure 2.15. Two “different” sizes for a porous particle

Particles with interconnected pores produce a size related to their solid volume not to their "envelope" volume (Fig. 2.15). The electrolyte is able to fill the pores allowing the current to pass through the particles and therefore produce smaller pulses from the aperture. There are several techniques to solve this problem. They are based on the ability to fill the pores with an organic substance, usually a solvent, solid at room temperature. For routine analyses, an initial assessment of the "envelope" size may be achieved by other methods, i.e. microscopy. As a result a correction factor is obtained and by entering this factor in the software it will correct the results to yield the "envelope size instead of the "solid" size. In quality control it is not important to know the "envelope" size. The specification of the product can be referred to the "solid" size and for practical purposes it will make no difference in quality control.

3. Measurement Precision

In order to achieve maximum measurement precision, there are two potentially competing factors that must be considered. First, the total number of particles counted must be sufficiently high and second, the concentration of particles must be such that coincidence limitations are not exceeded. In other words, ideal counting conditions exist with large measurement volumes of low particle concentration. Generally these conditions are neither practical nor are they necessarily achievable. It is, however, important that any particle counter/sizer should measure a large number of particles in order to achieve the greatest statistical confidence in the result. When it is not possible to accumulate a large number of counts i.e. when analyzing clean fluids, it is advisable to run the analysis in triplicate and use the mean value obtained from the three runs.

Table 2.3 presents precision statistics obtained from experimental data from 31 measurements of 10 μm latex particles.

Average Count (n = 31)	SD (n-1)	CV %
106	9.46	8.92
1,076	30.8	2.86
9,755	85.8	0.88
10,005	85.0	0.85
10,500	99.4	0.95
49,444	221	0.45
98,559	234	0.24

Table 2.3. Precision of Particle Counts at Various Count Levels

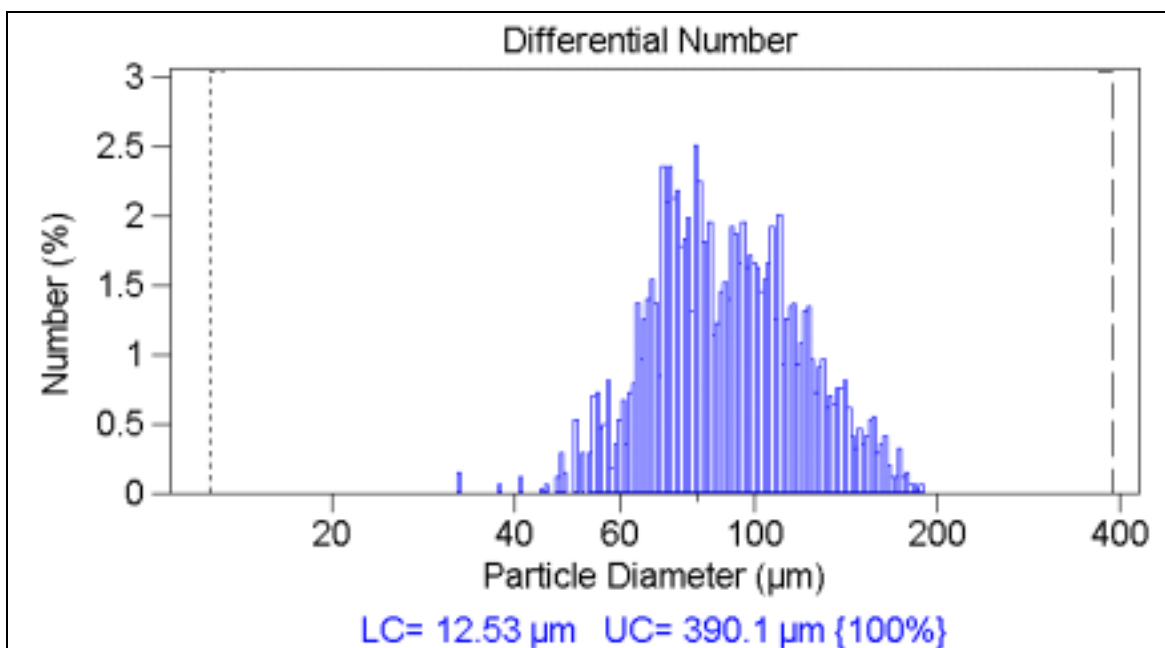


Figure 2.16. Few Particle Counts

Good statistics are of course indicated when there is little raggedness in consecutive channels in the DIFF mode, and when there is an absence of a regular rise in the data in the largest channels in the DIFF VOL mode, indicating that only one particle has been found in each channel. Fig. 2-16 illustrates this effect of insufficient particle counts; Fig. 2-17 the correct analysis after accumulating more data.

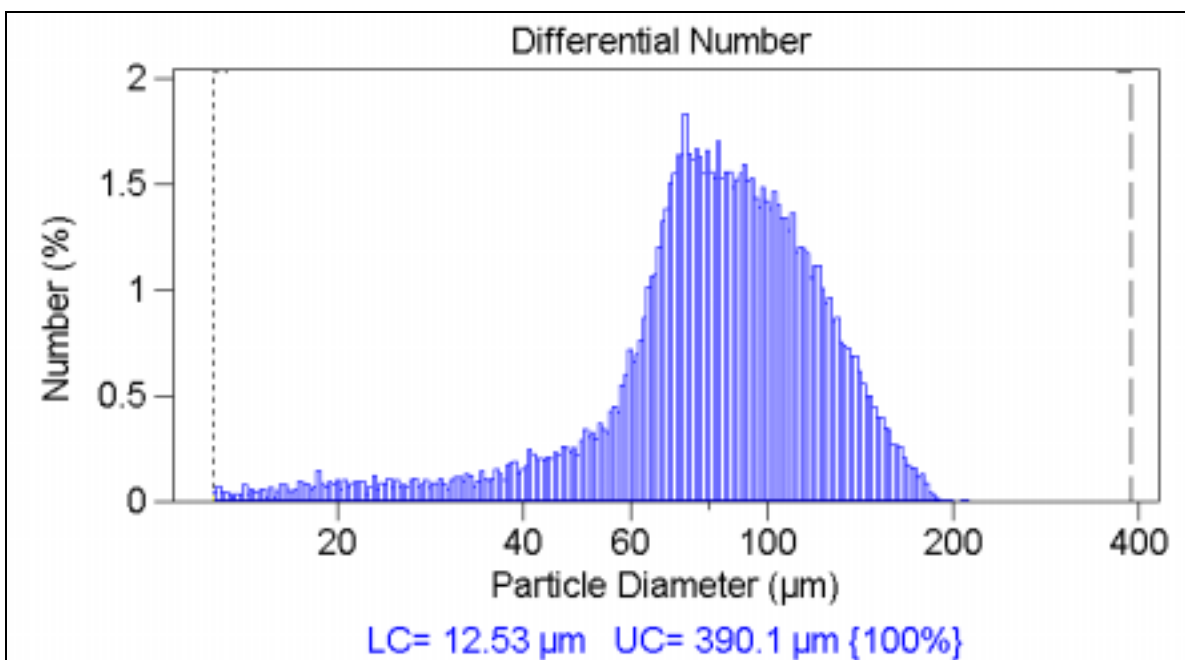


Figure 2.17. Correct Analysis after Accumulating More Data

4. Special Considerations For Using Small And Large Apertures

When using small apertures (20, 30 and 50 μm) or large apertures (400, 560, 1000 and 2000 μm), special care have to be taken due to their particular characteristics.

4.1. Using Small Apertures ($\leq 50 \mu\text{m}$)

Typically, 800 μA should be regarded as the maximum working current. Though there will be circumstances where 1 000 μA on a 30 μm aperture and 1600 μA on a 50 μm aperture will be acceptable. The aperture current should never exceed 800 μA with apertures smaller than 30 μm .

The main point to remember about small apertures is that the background count should be as low as possible, since the instrument is working near the limits of its capability. There are several sources of high background noise indicated below.

Dirty Electrolyte Solution. The factor, which influences the successful use of small apertures more than any other, is the strength and cleanliness of the electrolyte solution. Filtration of the electrolyte solution is of extreme importance.

For most electrolyte solutions, it will be found that increasing the salt concentration will increase signal to noise ratio at sizes below 1 μm .

For example 2-4% NaCl instead of 1% NaCl
 4 or 6% Na_3PO_4 instead of 2% Na_3PO_4 .

It should be noted, however, that an increase in electrolyte solution concentration may have an adverse effect upon the material being analyzed, and at all stages this should be checked.

When deciding upon the concentration of the electrolyte solution, it should be remembered that one of the limiting factors is the aperture resistance. Ideally the aperture resistance of all orifice tubes should be in the range 1 -1 100 K Ω .

Environmental Interference. It will be virtually impossible to obtain any satisfactory counts if the instrument is located in a dusty environment. The instrument should preferably be kept in either a reasonably dust-free room or enclosed in a clean air cabinet.

Electrical Interference. Any form of electrical interference will cause an increased background count. The most obvious symptom of electrical interference is noticed at the Concentration Index, it will tend to increase its reading and may also be unsteady. Any deviation from the normal should be immediately obvious. Interference may be eliminated by moving the instrument to a different power supply or by installing a voltage conditioner to suppress electrical noise.

Stirrer Motor. Another source of noise can be the stirrer motor. When using the instrument at 2% of the aperture diameter it may help to turn the motor off while performing the analysis. (Small particles will not settle out in this short time).

Damaged Aperture Wafers. A further possible source of noise is the orifice tube itself; this will normally only show at the smallest size levels as variable noise on a blank electrolyte solution, where none had been present before. If the orifice tube is the cause, then the blank count will tend to differ considerably from one aperture current polarity to the other. Fit another orifice tube with the same aperture diameter into the stand, and repeat the test.

Excessive Aperture Current. Excessive current causes the electrolyte solution in the aperture to heat, or even to boil, causing noise. This can normally be seen on the aperture-viewing monitor as either a dark plume of fluid or as bubbles leaving the aperture. The cure is to reduce the current or to increase the conductivity of the electrolyte solution. Prolonged boiling will permanently damage the aperture wafer.

Non Aqueous Electrolytes. When using organic solvents with low evaporation temperature, the current in the aperture may heat the solvent and cause electrical noise. This can be reduced by choosing a lower current setting and also by lowering the temperature of the electrolyte.

External Sound and Mechanical Vibration With the higher current densities that are encountered with small apertures, the instrument can be affected by either vibration of the supporting bench or by local sounds of high intensity (banging, shouting, etc) and these should be eliminated during the analysis.

4.2. 20 μ m Aperture.

When using the 20 μ m aperture to measure particles with a diameter approaching the lower limit of the instrument (approximately 0.4 μ m), all factors regarding the elimination of noise sources assume an even greater importance, and other factors are introduced. It is therefore recommended that the procedure adopted to measure these small diameters take into consideration the following points:

- (a) The electrolyte solution should be membrane filtered twice at 0.2 μ m and, where possible, should consist of a 4% saline solution or one of equivalent conductivity.
- (b) Ensure that the aperture current never exceeds 800pA. High currents can erode the aperture as well as generate electrical noise.
- (c) Computer monitors should be positioned as far as is practically possible from the instrument.

4.3. Using Large Apertures ($\geq 400 \mu$ m)

Using normal electrolytes may cause turbulence that can interfere with the analysis. Turbulence due to fast flow of low viscosity electrolyte solutions through the 2000, 1000, 560 and sometimes 400 μ m orifices can create an audible noise that can actually be heard as a 'whistle' and can be picked up as 'noise' on the instrument. Increasing the viscosity or density of the electrolyte solution substantially overcomes this problem and helps also to suspend dense particles. Viscosity should not generally be higher than the equivalent of 70% glycerol, usually an addition of 40% is sufficient.

The 400 μ m aperture design and configuration produce a 'border line case' i.e., some aperture tubes require the addition of glycerol to the electrolyte solution to prevent noise while others function perfectly well with low viscosity electrolyte solutions, for example ISOTON II diluent. It must be emphasized that nothing is physically wrong with a new 400 μ m aperture tube that requires an electrolyte solution of increased viscosity. In fact the addition of glycerol has a two-fold effect; by increasing viscosity the turbulent flow is reduced and at the same time the suspending of large, dense, particles is facilitated.

The upper size limit obtainable with 1000 μ m and 2000 μ m aperture tubes are very dependent upon the density of the material being analyzed and the practical upper limit of measurement is in the order of 1200 μ m with particles of density approximately 1 g/ml.